REVIEW ARTICLE

Sex Hormones, Immune Responses, and Autoimmune Diseases

Mechanisms of Sex Hormone Action

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Immune reactivity is greater in females than in males. In both experimental animals and in man there is a greater preponderance of autoimmune diseases in females, compared with males. Studies in many experimental models have established that the underlying basis for this sex-related susceptibility is the marked effects of sex hormones. Sex hormones influence the onset and severity of immune-mediated pathologic conditions by modulating lymphocytes at all stages of life, prenatal, prepubertal, and postpubertal. However, despite extensive studies, the mechanisms of sex hormone action are not precisely understood. Earlier evidence suggested that the sex hormones acted via the thymus gland. In recent years it has become apparent that sex hormones can also influence the immune system by acting on several nonclassic target sites such as the immune system itself (nonthymic lymphoid organs), the central nervous system, the macrophage-macrocyte system, and the skeletal system. Immunoregulatory T cells appear to be most sensitive to sex hormone action among lymphoid cells. Several mechanisms of action of sex hormones are discussed in this review. The possibility of using sex hormone modulation of immune responses for the treatment of autoimmune disorders is a promising area for future investigation. (Am J Pathol 1985, 121:531–551)

MANY DECADES ago scientists and clinicians alike observed that there were striking differences between the immune responsiveness of males and that of females. In general, females had superior humoral and cell-mediated immunity. Simultaneously, clinicians noted that women were more resistant to a variety of infections, which correlated with their greater longevity. Although these observations implicate the influence of sex-hormonal factors, these aspects received relatively scant attention. In subsequent years, when the concept of immunology advanced from a mere response to infectious agents to include a variety of complex situations such as loss of self-tolerance and aberrant responses to self-antigens (autoimmunity), it became apparent that women also had exaggerated responses to autoantigens and hence were much more susceptible to autoimmune diseases. Again, rather surprisingly, studies on the influence of sex factors were largely ignored. However, in comparatively recent times, several reports documenting the influence of sex factors (sex hormones) in a variety of animal models of autoimmune diseases have rejuvenated an interest in this area. This review will 1) summarize the past and present state of knowledge in the area of sex hormones and the immune system; 2) present pertinent problems associated with sex hormonal studies; 3) propose explanations for the conflicting reports on the immunologic effects of sex hormones; 4) offer possible mechanisms of sex hor-

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mone action on the immune system in general and autoimmunity in particular.

Space limitations permit us to discuss only selected studies. In the main, major emphasis on the effects of sex hormones are given to two different models of autoimmune disease: 1) spontaneous lupus in NZB/NZW (F_1)[(B/W)] mice[1,2]; and 2) autoimmune thyroiditis induced in normal rats following early thymectomy and a series of sublethal irradiation (Tx-X).[3,4] These studies will form the basis to compare and contrast with other experimental and human situations.

Sex Differences in Heteroimmune Responses

It has become increasingly apparent that females have better immune capabilities than males.[5-7] They have higher immunoglobulin levels—IgM,[8-10] IgG,[5,11-13]—and stronger humoral and cell-mediated immune responses than males. This is evident from their superior responses to a variety of antigens,[5,13-15] their ability to reject allografts more rapidly,[16-18] better *in vitro* response to mitogens and other *in vitro* immunologic assays,[19-22] and relative resistance to the induction of immune tolerance.[23] Females also tend to have a reduced incidence of certain tumors[24-26] and a higher incidence of regression of induced tumors,[24] and generally resist a variety of bacterial and viral infections and parasitic infestations more successfully than males.[27-36] Cytotoxicity to certain viruses is much greater in females than males.[37] Furthermore, the survival rate of females is greater than males, presumably because of their better immune capability.[27,28,38-42]

Sex Differences in Autoimmune Diseases

In Humans

A differential sex susceptibility to various autoimmune diseases has long been recognized. In general, women have a greater propensity for the occurrence of these diseases (Table 1). For example, the female-to-male susceptibility ratio in systemic lupus erythematosus (SLE) and the adult-onset form of Hashimoto thyroiditis is 9:1[48] and 25-50:1, respectively.[43-45] Furthermore, independent surveys of normal populations revealed that there is a higher prevalence of autoantibodies to thyroid components in women than in men of the same age.[60,61]

In Animals

A similar sex-related expression of disease is known to occur in several animal models of autoimmune diseases (Table 2). In particular, female B/W mice have an accelerated expression of lupus and Sjögren's syndrome-like diseases, compared with males.[73,83,84] In these mice severe immunocomplex glomerulonephritis, autoantibodies to nucleic acids, and proteinuria develop.[70,73,83] Kidneys are the primary tissue of autoimmune attack in murine SLE. Grossly, kidneys are enlarged and discolored. The immune complex deposition on the glomeruli is more diffuse and of greater fluorescent intensity in females than in age-matched males. The immunoglobulin deposition involves both the mesangium and the capillary basement membrane. Early renal lesions, evidenced by light microscopy, are

Table 1—Female Preponderance of Autoimmune Diseases in Humans

Diseases	Female-to-male ratio	References
Thyroid diseases		
Diffuse lymphocytic thyroiditis		
Goitrous, struma lymphomatosa (Hashimoto)		
Hypercellular variant adult onset	25–50:1	43, 45
Juvenile onset	4–7:1	44, 46, 47
Fibrous variant	4:1	44
Nongoitrous		
Severe atrophic (myxedema)	6:1	44, 45
Mild atrophic (asymptomatic)	8:1	44
Primary hyperthyroidism (Graves-Basedow disease)		
With benign or no exophthalmos	4–8:1	44, 45
With progressive ophthalmopathy	2:1	44
Systemic lupus erythematosus	9:1	48–50
Rheumatoid arthritis	2–4:1	51, 52
Sjögrens' syndrome	9:1	53
Idiopathic adrenal insufficiency (autoimmune adrenal disease)	2–3:1	45, 54
Scleroderma	3–4:1	55, 56
Myasthenia gravis	2:1	57
Multiple sclerosis	1–5:1	58
Autoimmune diabetes mellitis (Type 1b—Insulin dependent diabetes mellitus)	5:1	59

characterized by endothelial and mesangial hypercellularity, followed by immune complex and hyaline deposition, deformity of the capillary basement membrane, and absence of epithelial crescents. Females have an accelerated acute glomerulonephritis, which is frequently associated with proliferation of renal capsular cells and fibrinoid necrotic changes, compared with age-matched males. Electron-microscopic examination of kidneys demonstrated electron-dense deposits in the intracapillary and epithelium of the basement membrane of glomeruli of young adult females.

Furthermore, B/W mice also develop Sjögren's syndrome-like disease, as is often seen in human SLE patients. The intensity of the disease is more severe in females, compared with males. There are immunopathologic changes in the lining of ducts and of lobules of acini, with periarteriolar and periductal and interstromal and intrastromal lymphocytic infiltrations in the submandibular, salivary, and lacrimal glands.

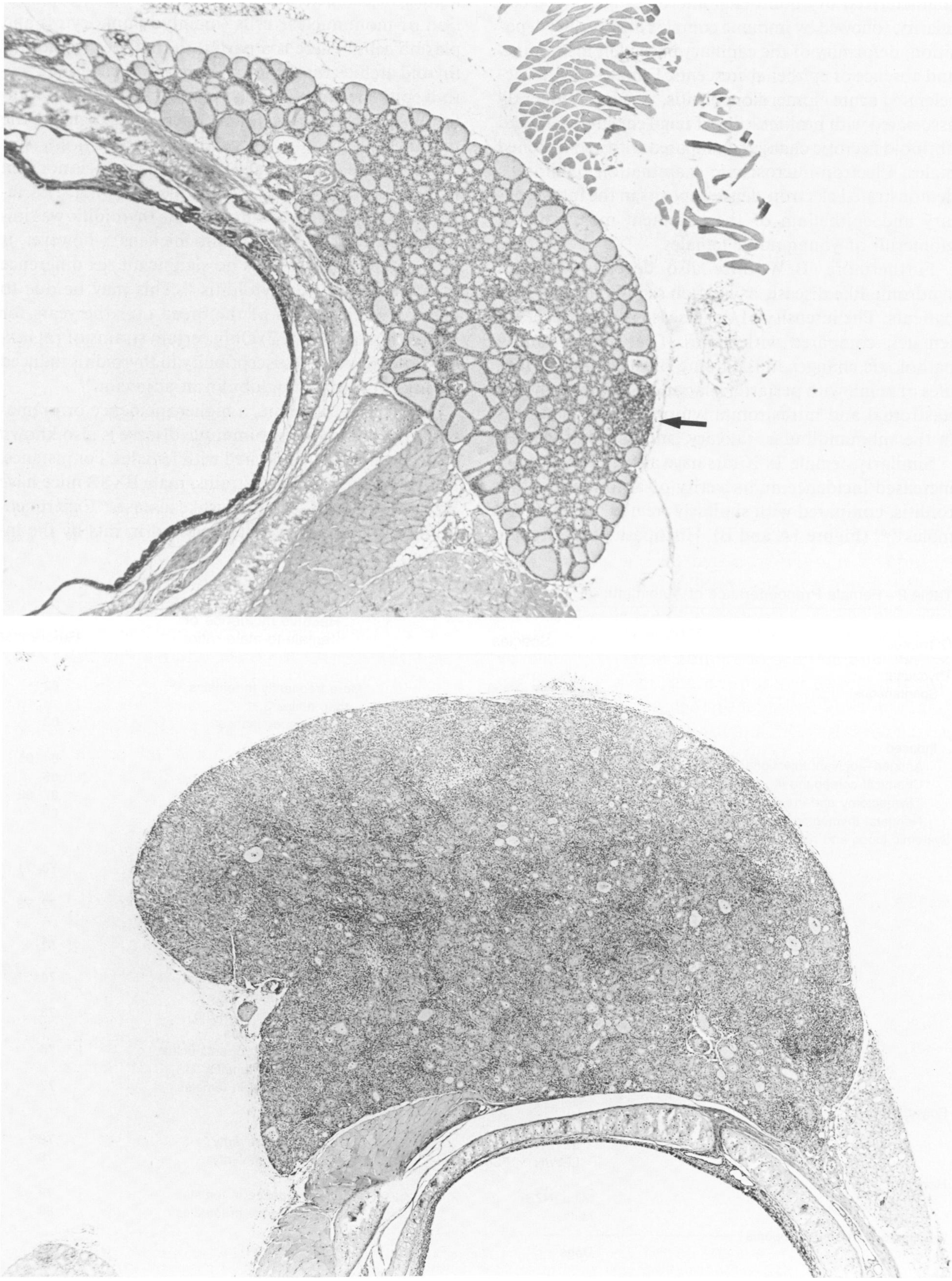
Similarly, female Tx-X rats have a three to five times increased incidence and severity of autoimmune thyroiditis, compared with similarly treated age-matched males[67,68] (Figure 1A and B). Histopathologically, the lesions are characterized by moderate to massive infiltration of mononuclear cells (mainly lymphocytes) and plasma cells. There is a partial to total destruction of thyroid architecture (graded from 0 to 4), including thyroid epithelial and follicular cells and colloid. This autoimmune pathologic lesion does not extend to the parathyroid.

The female predominance, although usual, is not seen in all models. Examples include the following: 1) A female predominance of autoimmune thyroiditis was initially observed in obese strain chickens[63]; however, it now appears that there is no significant sex difference in susceptibility to thyroiditis.[85] This may be due to genetic manipulation of the breed over the years for higher susceptibility. 2) Only certain strains of rats exhibit the sex-related susceptibility to thyroiditis induced by injection of thyroglobulin in adjuvant.[64]

At the other extreme, a higher incidence or premature appearance of autoimmune disease is also known to occur in males, compared with females. For instance, unlike other SLE-prone strains, male BXSB mice have an earlier onset of severe lupuslike disease.[86] Experimental autoimmune thyroiditis induced in rats by the in-

Table 2—Female Preponderance of Autoimmune Diseases in Animals

Diseases	Species	Relative incidence or female-to-male ratio	References
Thyroiditis			
Spontaneous	Rats (BUF strain)	More frequently in females than males 3:1	62
	Chicken (OS strain)	Female predominance	63
Induced			
Antigen–adjuvant injections	Rats	2:1	64, 65
Chemical compound ingestion	Rats	2:1	66
Thymectomy and irradiation	Rats	4-6:1	67, 68
Neonatal thymectomy	Mice	4:1	69
Systemic lupus and rheumatoid arthritis			
	Mice (strains)		
	NZB	Earlier appearance of the disease in females	70, 71
	NZB/NZW Fl	Earlier appearance of the disease in females	72, 73
	NZB/DBA/2 Fl	Earlier appearance of the disease in females	74
	NZB/CBA/N Fl	Earlier appearance of the disease in females	74
	MRL/lpr and MRL/n	Earlier appearance of the disease in females	75
	C57BL/6-lpr	Higher incidence of antinuclear antibodies in females	76
	Dogs	Higher incidence in females	77
Polyarthritis			
	Rats		
	LEW/N	6:1 severity	78
	LEW/N × F344/N FL	3:1 severity	78
Hemolytic anemia			
Spontaneous	Mice (NZB)	Higher incidence in females	79
Induced	Mice	Higher incidence in females	80
Autoimmune thrombocytopenia			
	Dogs	2:1	81 82



A

B

gestion of the immunosuppressive compound frentizole occurs much more frequently in males.[87] In humans, eosinophilic fasciitis of probable autoimmune origin occurs twice as often in males as in females.[88]

In any event, apart from the above few exceptions, evidence presented in Tables 1 and 2 strongly demonstrates a preferential susceptibility of females to autoimmune diseases in both humans and animals.

Basis for Sex Differences in Heteroimmune and Autoimmune Responses

The mechanisms underlying this differential response are imperfectly understood. In this respect, the chief possibilities are 1) a direct control exerted by an X or a Y chromosomal gene(s); and 2) an indirect modulatory influence of sex steroids. Attempts to explain this effect solely on the basis of sex chromosome genes have been equivocal. IgM, but not IgG, levels have been correlated with the number of X chromosomes.[12,89,90] However, in other studies in humans and mice, such an association was not readily apparent.[91]

Sex chromosomes have also been linked to the expression of immune-mediated disorders. For example, the accelerated susceptibility of lupus in BXSB males is due to the Y chromosome and not due to differing sex hormonal levels.[86,92] Furthermore, in experimental autoimmune thyroiditis induced in rats by thyroglobulin-adjuvant injections, the sex-related autoimmune response to rat thyroglobulin is found to be associated with the X chromosome.[64] However, sex hormone manipulations were not performed in these studies, and hence the effects of hormonal agents cannot be conclusively determined. In humans, males bearing X-linked lymphoproliferation gene are predisposed to Epstein-Barr virus infections, and in them a variety of lymphoproliferative disorders develop, such as malignant lymphomas and malignant mononucleosis.[93]

On the other hand, sex hormones have been implicated to explain the majority of sex differences in immune responses. For example, orchiectomy has been found to augment the immune response to a variety of antigens.[5,13,94] Furthermore, extensive studies in many laboratories have shown that sex hormones have a profound influence upon the immune system.[7,95-99] This aspect is addressed in more detail in the subsequent section.

It is probable that the higher incidence of various autoimmune disorders in women indirectly reflects the effects of sex hormones. There is some presumptive evidence to support the proposition that sex hormones may influence the pathogenesis of clinical autoimmune diseases. For example, abnormal estrogen metabolism resulting in prolonged estrogenic stimulation has been reported in patients with SLE[100] as well as those with Klinefelter's syndrome, in whom SLE frequently develops.[49] This aberrant sex hormone metabolism is believed to influence the course of autoimmunity.[49] Furthermore, the total serum androgen concentration is also reduced in SLE patients.[101] The fact that Klinefelter's syndrome patients have poorly developed testes and male genitalia is again strongly suggestive of sex-hormonal involvement. Hormonal changes during pregnancy or at menarche are believed to modulate several autoimmune diseases, such as SLE,[102-105] autoimmune thyroiditis,[106] Graves' disease,[107] rheumatoid arthritis,[108] idiopathic thrombocytopenic purpura,[109] and polymyositis/dermatomyositis.[110] It is noteworthy that male Graves' disease patients with gynecomastia have an imbalance between estrogen and androgens.[111] However, it is not known what effects these have on the pathogenesis of the disease.

The role of sex hormones in autoimmune diseases has been further strengthened by the clinical observation that the administration of oral contraceptives altered the course of many autoimmune diseases in humans.[104,105,112-115] For example, administration of estrogen-containing oral contraceptives to lupus patients was associated with exacerbation of the disease. Withdrawal of the contraceptive pill resulted in the amelioration and remission of the disease. Interestingly, in clinically healthy women on oral contraceptives, with no history of autoimmune diseases, LE cells characteristic of lupus[113] or rheumatic symptoms developed.[114] Furthermore, one report documented that in apparently healthy women lupus developed following oral contraception.[115] Although the precise influence of oral contraceptives in the development of autoimmunity in normal individuals is not known, it is likely that in most individuals, these agents may be "harmless" in terms of induction of autoimmune diseases. Nonetheless, susceptible but asymptomatic individuals (with predisposing genetic and viral components) may be pushed over a protective brink by sex hormones into a stage of clinical disease expression.

Further suggestion of sex-hormonal involvement in

Figure 1A—H&E section of the thyroid of a thymectomized and irradiated intact male rat. Arrow indicates a small focus of mononuclear cell infiltration (score 1+). (×48) **B**—H&E section of the thyroid of a thymectomized and irradiated intact female rat. Note the extensive pathologic changes characterized by massive infiltration of lymphocytes and destruction of thyroid architecture (score 4+). (×48)

Table 3—Role of Sex Steroids in SLE-Like Disease in B/W Mice

1. B/W females have an accelerated SLE-like disease and die earlier, compared with males.
2. Sex hormones influence survival time in B/W mice. At 8 months of age, there was 100% mortality in estrogen-treated male mice, while in androgen-treated male mice there was only 10% mortality.
3. Estrogen enhanced the IgM and IgG autoantibodies to DNA, while androgen reduced these autoantibodies.
4. Estrogen also enhanced IgM and IgG autoantibodies to poly-A. In contrast, androgen suppressed the levels of these autoantibodies.
5. Estrogen augmented glomerulonephritis, while androgens had an ameliorative effect.
6. Androgens were beneficial even in animals with established autoimmune disease, as measured by survival, autoantibodies, and renal disease.

Original data obtained from Roubinian et al.[73] and Roubinian et al[83]

autoimmunity includes the inverse relationship of the development of the antinuclear and anti-smooth-muscle autoantibodies with serum testosterone levels in male patients with alcohol-induced cirrhosis.[116]

Evidence in the experimental situation has been more direct. Thus, the differential expression of spontaneously occurring lupuslike syndrome in B/W mice has been shown to be the consequence of the effects of sex hormones, since alteration of sex hormone levels either by orchiectomy or sex hormone replacement markedly influence the pattern of the disease. Prepubertal orchiectomy of B/W mice caused an earlier expression of the disease and shorter life span, comparable to that of females. Administration of androgen significantly delays this process.[70,73,117–120] Androgens were of therapeutic value in both sexes even at a time when SLE was already well established (6 months of age) (Table 3).[121] In contrast to orchiectomy, ovariectomy had little influence on the disease process. However, administration of estrogens or progesterone accelerated the disease process.[117] There was exacerbation of glomerular immunoglobulin deposition in estrogen-, but not androgen-treated male and female mice. Estrogen-treated mice showed electron-dense deposits in the mesangial area of the glomeruli. In contrast, no such deposits were seen in androgen-treated mice. These results in B/W mice generated significant interest in the area of sex hormones and autoimmunity.

The male resistance to the expression of thyroiditis in Tx-X rats is also due to the presence of the testes, since orchiectomy greatly increased the incidence and severity of thyroiditis to the level which occurs in females.[67,68] Replacement of testosterone in orchiectomized or female Tx-X rats suppressed thyroiditis expression which was dose-dependent[122] (Table 4). Testosterone administration was also found to be of therapeutic value in Tx-X rats with established thyroiditis.[123] The thyroiditis-positive animals selected for hormonal therapy were confirmed by transplantation of

Table 4—The Effect of Testosterone in Oil on the Development of Thyroiditis in Orchiectomized Tx-X Rats

Group	Number of animals	Thyroiditis: Incidence (%)	Thyroiditis: Mean thyroid pathology ± SE	Antibodies to thyroglobulin: Incidence (%)	Antibodies to thyroglobulin: Mean antibody titer (log) ± SE
1) Sham-orchiectomized	11	18	0.4 ± 0.2	18	1.7 ± 1.1
2) Prepubertal orchiectomized	26	73	1.7 ± 0.2	80	6.2 ± 0.8
3) Prepubertal orchiectomized + oil	7	71	1.9 ± 0.6	85	7.0 ± 1.7
4) Prepubertal orchiectomized + testosterone in oil					
a) 150 ng	20	40	0.7 ± 0.2	45	2.7 ± 0.8
b) 1 μg	8	38	0.7 ± 0.4	38	3.5 ± 1.9
c) 150 μg	12	33	0.6 ± 0.4	41	3.5 ± 1.4
d) 500 μg	10	0	0	10	0.4 ± 0.4
e) 5000 μg	9	0	0	0	0

Numbers in each group are generally the total of several experiments with a minimum of 8 animals per experiment.

Statistical significance (Student *t* test)

Group	Thyroiditis	Autoantibodies
1 vs 2	$P < 0.01$	$P < 0.01$
2 vs 3	NS	NS
2 vs 4a	$P < 0.05$	$P < 0.01$
2 vs 4b	NS	NS
2 vs 4c	$P < 0.05$	$P < 0.05$
2 vs 4d	$P < 0.001$	$P < 0.001$
2 vs 4e	$P < 0.001$	$P < 0.001$

From Ansar Ahmed and Penhale.[122] By permission.

syngeneic normal thyroids under the renal capsule of Tx-X animals for 4 weeks followed by its surgical removal for histopathologic processing. Our earlier studies had shown that the incidence and extent of thyroiditis in the grafts precisely correlated with findings in the animals' own (native) thyroids.[124] Testosterone therapy of autoimmune thyroiditis-positive animals for 2 months caused complete regression of the lesions, although autoantibodies to thyroglobulin remained unaltered. Ovariectomy of Tx-X rats further enhanced thyroid autoreactivity, compared with rats that underwent sham operations or untreated control rats.[68,125] Estrogen, but not progesterone, suppressed thyroiditis (Table 5).[125] Although the precise reason for female hyperactivity is not readily apparent, it is believed that this may be due to low levels of immunosuppressive male sex hormones or an imbalance of progesterone and estrogen ratios.[125] The direct influence of sex chromosomes has been discounted in this model.[68]

Effects of Sex Hormones on Heteroimmune Responses

Surgical ablation of testes has been found to result in immunopotentiation in many systems. For example, orchiectomy increased protection against viral and fungal[7,97,30,32] and bacterial infections[7] and parasitic[126] infestations. Also, orchiectomized animals reject allografts rapidly[17,94] and have accelerated graft-versus-host reactions.[94] Furthermore, gonadectomy augmented T-lymphoblast transformation in culture[127] and potentiated humoral immune response to several heteroantigens.[5,13,94] However, in some experimental situations, orchiectomy had no effect on the immune system.[7]

The administration of testosterone has been shown to reduce both humoral and cell-mediated immune responses.[97,128–131] However, in other systems testosterone had either no effect or enhanced immune responses.[7,96,129]

Endocrine changes during pregnancy (increased progesterone to estrogen ratio) may be responsible for a moderate degree of immunosuppression sufficient to prevent the rejection of the fetus which is a semiallogeneic graft. In particular, cell-mediated immune functions are generally depressed. High concentrations of naturally occuring placental progesterone are immunosuppressive in a number of immunologic *in vitro* assays.[132–135] However, it must be borne in mind that several placental products and other pregnancy-associated factors are also immunosuppressive.[132,136–138]

Studies on the effects of female sex hormones on the immune system in which different experimental situations were employed have yielded conflicting results. The potentiating effects of ovariectomy on the immune response to several antigens have been observed in normal animals, which suggests that ovarian hormones may be immunosuppressive. The ovariectomized animals have better protection against a variety of infections,[30,32,126] as well as induction of tumors.[139] Conversely, ovariectomy also depressed humoral response to heteroantigens[140,141] and did not result in a proficient defense against certain infectious agents.[29,32,34]

Estrogen has been shown to suppress both humoral and cell-mediated immunity as well as enhance immune responses in several systems.[5–7,14,142–147]

As with the effects of estrogen on the immune system, the precise effects of progesterone are unclear. This hormone has been shown to suppress as well as enhance immune responses.[131–134,148] Progesterone prolongs allogeneic and xenogeneic grafts in several species,[149–151] suppresses mitogenic responses *in vitro*,[131,134,151–152] generates suppressor cells,[153] and promotes the incidence of tumors.[139] However, in other studies progesterone failed to alter the survival time of skin graft[154] or mitogenic responses.[131] Progesterone and estrogen have been shown to exert opposite effects in several studies. For example, progesterone, but not estrogen, levels correlated with high antibody titers to *Candida albicans*,[148] and progesterone administration has been found to afford protection against viral infections, as opposed to the increased susceptibility following estrogen administration.[155] A higher incidence of induced tumors was observed in ovariectomized animals treated with estrogen but not with progesterone.[156]

Table 5—The Effect of Estrogen in Oil on the Development of Thyroiditis in Orchiectomized Tx-X Rats

Groups	Number of animals	Incidence (%)	Mean thyroid pathology ± SE	Incidence (%)	Mean titer (log) ± SE
1) Orchiectomized	8	100	2.1 ± 0.3	100	7.6 ± SE
2) Orchiectomized + estrogen	8	25	0.6 ± 0.4	38	2.5 ± 1.2

Statistical significance	Group	Thyroiditis	Antibodies
Incidence (Fisher's exact)	1 vs 2	$P < 0.005$	$P < 0.05$
Severity (Student t test)	1 vs 2	$P < 0.01$	$P < 0.005$

From Ansar Ahmed et al.[125] By permission.

Effects of Sex Hormones in Other Autoimmune Diseases

The effects of sex hormones in several other autoimmune diseases are now known. The inhibitory effects of sex steroids on autoimmune diseases were initially demonstrated in experimental autoimmune thyroiditis induced in guinea pigs and rats by thyroid extract adjuvant administration.[157] Both testosterone and estrogen at moderately high doses suppressed autoimmune thyroiditis in guinea pigs. Similar effects of testosterone, but not estrogen, were noted in autoimmune thyroiditis induced in rats.[157]

Similarly, the course of experimental autoimmune thyroiditis induced in mice by exogenous thyroglobulin injections is also modulated by sex hormones.[158] Orchiectomy or estrogen treatment of good responder mice C_3H augmented autoantibodies to thyroglobulin. Testosterone adminstration reversed this effect. However, no significant modulatory effects of sex hormones were observed in poor responder Balb/c mice. In any event, in this model the effect of sex hormones was seen only on autoantibodies and not on thyroid lesions.

The ameliorative effects of androgens has also been demonstrated in MRL/lpr mice which develop a very aggressive lupus and lymphoproliferative disorder.[159,160] Treated mice have reduced autoantibody levels and improved renal function and survival time but without any significant effects on lymphoproliferation.[159]

Testosterone reduced, while estrogen enhanced, the spontaneous autoantibody production in NZB × C_3H,[70] NZB × CBA,[70] NZB × SJL/J (B/S),[161] and NZB × DBA/2 (B/D) mice.[74] Furthermore, it has recently been shown that administration of dehydroisandrosterone (a proandrogen) to B/W mice delayed the onset of lupus.[162]

The resistance of male LEW/N rats to induced polyarthritis by the injection of peptidogylcan-polysaccharide fragments is due to the presence of male sex hormones, since orchiectomy or estrogen treatment renders them susceptible to the disease. Estrogen treatment rendered the resistant strain, F344/N, susceptible to polyarthritis. Genetic studies established the lack of influence of sex chromosomes on the disease process.[78]

Autoantibodies to acetylcholine receptor in experimental autoimmune myasthenia gravis induced in susceptible mice by acetylcholine receptor in complete adjuvant injections are also altered by orchiectomy or ovariectomy (P. Christadoss, S. Ansar Ahmed, T. Lindstrom, S. Munroe, N. Talal, manuscript in preparation) or by the administration of sex hormones[120] (P. Christadoss, S. Ansar Ahmed, T. Lindstrom, S. Munroe, N. Talal, manuscript in preparation). Orchiectomy increased, while ovariectomy or testosterone administration reduced autoantibodies to acetylcholine receptor.

The development of autoimmune hemolytic anemia induced in mice by xenogeneic erythrocyte immunization is also altered by sex hormone manipulation.[80] For example, testosterone had an inhibitory effect on the disease.

Thus, it appears that in most cases, physiologic endogenous sex hormones, rather than sex chromosomes, contribute to the susceptibility or resistance to autoimmune diseases.

Exogenous estrogen (oral contraceptives) inhibited induced experimental allergic encephalomyelitis in rats, presumably by depressing T-cell–mediated functions,[163] again demonstrating the modulatory influence of sex hormones.

The experimental findings of suppression of autoimmune disease by male sex hormones has now led to clinical therapy trials in humans. For example, danazol, an attenuated male sex hormone, has been successfully used in the treatment of female-predominant idiopathic thrombocytopenia[164] and idiopathic thrombocytopenia associated with SLE.[165] Similarly, clinical studies in B/W mice using weak androgenic compounds revealed that nandrolone (but not danazol) had ameliorative effects on autoimmunity.[84,166]

Taken together, the above studies imply that male sex hormones consistently protect against several types of autoimmune diseases which presumably differ in their induction and pathogenic pathways. On the other hand, the effects of estrogen appear to vary among the models, although, in general, estrogen promoted many autoimmune diseases.

Possible Reasons for Conflicting Results

The contradictory nature of the data on the effects of sex hormones on the immune system both in normal and autoimmune situations are the consequences of many variables.

Dose of Hormones

Since the lymphoid cells of rodents in general, and of the mouse in particular, are considered to be highly sensitive to the effects of sex hormones,[70] any variations in the dose of hormones could lead to different results. Thus, it has been shown by earlier workers that low dose of estrogen did not prolong the incompatible skin grafts in rodents,[167,168] but high doses did have this effect.[169] More recently, it has been shown in autoimmune responses in murine lupus that even small variations within the physiologic range can elicit different results.[70] It is for these reasons a wide dose range of hormones was employed to study their effect on the development of autoimmune thyroiditis in Tx-X rats. Although there was some suggestion that estrogen at low doses may augment the severity of autoimmune thyroiditis and levels of autoantibodies to thyroglobulin in Tx-X, this was found not to be of statistical significance.[125] However, significant suppression of the disease was seen at relatively high doses.[125] An apparent dual effect on the type of autoimmune response by testosterone was not seen. The degree of suppression of thyroiditis in Tx-X rats by testosterone was found to be directly dependent upon the dose of the hormone.[122] Similarly, in our recent series of studies, we observed that the administration of sex hormones to normal mice brought about, in general, a dose-dependent degree of changes in sex-hormone-sensitive lymphocytes (Thy-l.2 and Lyt-2) in the thymus.[99]

Influence of "Sex Hormonal Genes"

Stern and Davidson[14] observed that the effect of sex hormone on the immune response to sheep red blood cells (SRBCs) varied with the strain of mice. Similarly, certain strains of autoimmune-prone mice respond poorly to sex hormones. For example, NZB mice, unlike its hybrids, are naturally resistant to testosterone.[70] In order to investigate the reasons underlying marked strain differences in response to the effects of sex hormones, we initiated a large series of experiments employing several B10 congenics and other inbred strains of mice on non-B10 background. Testosterone was given prepubertally to intact mice for 2–3 months, and immune response to T-dependent antigen purified protein derivative (PPD) was measured. Our results indicate that both genes within and outside the major histocompatibility complex (MHC), tentatively termed "Ir-Te genes," modulate the degree of this sex-hormone-influenced immune response (S. Ansar Ahmed, N. Talal, P. Christadoss, manuscript submitted). The genetic influence on the sex-dependent immune responses has been recently suggested in another study.[22] Spleen cells derived from female normal mice were found to be superior to those obtained from males in antigen presentation and mixed lymphocyte reaction, which are restricted by Ia and H-2 antigens. The underlying reasons have been attributed to the influence of male sex hormones. It is noteworthy that the effects of sex hormones may vary with the species (eg, humans and rodents may be more sensitive to sex hormone action, compared with other species).

The Age at Administration of Hormones

This may also be crucial in certain situations; eg, orchiectomy performed prior to puberty is more effective than that performed after maturity. The hormone dose found effective when initiated at early stages of murine lupus was found to be ineffective given at a later stage.[70] Furthermore, sex hormones given shortly after birth have much more pronounced effects on the immune system than when administered to weanling or adult animals. For example, neonatal estrogen administration to mice results in a condition resembling Runting syndrome, which eventuates in accelerated mortality.[142]

In our recent studies we observed that prenatal exposure of mice to sex hormones greatly affected the immune capability, as evidenced by permanent changes in lymphocyte subset numbers, IL-2 production, autoantibody production, and NK cell activity (S. Ansar Ahmed, M.J. Dauphinee, N. Talal, manuscript in preparation).

Route of Administration of Hormones

This may also be a contributory factor to the variability of the results. It has been shown that hormones in implants have a better effect than repeated injections, presumably because of sustained release of hormones through implants.[70] However, direct injection of hormones in oil base (for slow release of hormones) may be a better form of administration, because the dose of hormone given can be carefully controlled. A direct comparison of the relative effects on the immune system of sex hormones given orally with that given parentally is now known. It is likely that hormones given orally may act on gut-associated lymph nodes (mesenteric lymph nodes), whereas those given parentally may act on spleen and draining lymph nodes. Thus the initial immunologic events following exposure of hormones may differ, which could consequently affect the type or magnitude of immune response.

Time of Administration and Dose of Antigen Under Study

Different results have been obtained in relation to the time of administration of sex hormones and the antigen injection.[6,142] Furthermore, similar experimental protocols but variable doses of antigens have altered the pattern of results.[6,149,168] This could be due to variations in the antigen localization and the stimulation of the reticuloendothelial system, which plays an important part in immune response.

State of the Host

The effects of sex hormones are more pronounced in irradiated animals than in nonirradiated animals.[142] More recently, the inhibitory effect of testosterone on the immune response to SRBCs in autoimmune mice could only be demonstrated when testosterone was followed by sublethal irradiation. This suggests that testosterone acts on cells undergoing hemopoietic regeneration. Alternatively, the effects of sex hormones may be more marked in animals deprived of, or with low numbers of, radiosensitive cells. This may be one explanation for the differences in the type of effect of estrogen on thyroiditis in Tx-X rats and on lupus in B/W mice. Furthermore, the type or degree of effects of sex hormones on the immune system may vary in health (normal) and disease (autoimmune/lymphoproliferation) states.

Differential Response to Various Antigens

There is some suggestion of selectivity about the action of sex hormones. In this regard, it has been shown that testosterone administration can have different effects on various autoantibodies within the same animal.[70] For example, testosterone administration to NZB mice reduced anti-DNA antibodies but had no effect on erythrocyte antibodies and increased levels of thymocytotoxic autoantibodies. Furthermore, we have noticed that sex hormone (testosterone) administration to normal mice markedly inhibited responses to T-dependent antigens PPD and concanavalin A (Con A), but not T-independent antigens (mitogen response to lipopolysaccharide) within the same animal (S. Ansar Ahmed, N. Talal, P. Christadoss, manuscript in preparation). These observations imply that the effects of sex hormones on immune responses cannot be generalized and must be treated individually.

Conversion of Sex Hormones

Male sex-hormones such as testosterone may be converted to estrogen by peripheral aromatization in adipose, muscular, hepatic, placental, and other tissues, thus complicating interpretation. Thus, the fact that in some studies testosterone treatment resulted in enhanced immune responses may be due to the conversion to estrogen. It may be useful to employ 5-α-dihydrotestosterone, which is not converted to estrogen.[170]

Sham Surgery

In several studies sham surgical ablation of gonads have not been included. This is a necessary control, because stress can itself modulate the immune response.[171] Furthermore, the success of surgical ablation of gonads, particularly ovaries, at the termination of the experiment has not been reported in many studies. This is especially important because small ovarian tissue rudiments can regenerate with time.

Castration as a Means of Depleting Sex Hormonal Levels

Sex hormones are produced at different sites and are not exclusive to the gonads.[172] Gonadectomy reduces sex hormonal levels but does not totally deplete sex hormones. The function of gonads may be compensated by extragonadal tissues. Thus, the lack of effect of gonadectomy in certain situations may be due to adequate replenishment of sex hormones from extragonadal tissues. This problem emphasizes the need to determine serum sex-hormonal status following sex-hormonal manipulation.

Assay Variations

Variations in the effects of sex hormones may also be due to inherent differences in the assay system, the time of assay in relation to antigen immunization, and the type of assay system. Furthermore, even within the same animal, different immunologic assays may yield varied results, presumably due to the different degree or type of effects of sex hormones on the immune system.

Target Cells

The target cell for sex hormone action may greatly vary among different systems. Thus, the type of signal that lymphocytes receive (end target cell) may differ markedly in various experimental systems, which consequently affects the type of immune responses. This aspect is discussed in greater detail in the latter part of this article.

Sex Hormone Receptors

It is currently believed, although we are lacking in evidence, that sex hormones act on the lymphocytes via sex hormone receptors (SHRs). It is conceivable that there may be qualitative or quantitative differences in these receptors among different strains, which may account for variation in sex-hormone–influenced immune responses.

In conclusion, the subtlety and variability of effects of sex hormones on the immune system are clearly apparent. Thus, because of different experimental designs, approaches, and conditions employed by several investigators, it is difficult to arrive at an embracing consensus on mechanism of action. Nevertheless, it is clear that sex hormones have several different modes of activity, involving multiple sites, and may trigger cells with diverse functions.

Possible Mechanisms of Sex Hormone Action

Although the effect of sex hormones on the immune system has long been recognized, several questions regarding the precise mechanism of action have remained unanswered. These include 1) What organ or tissues are sensitive to sex hormones? 2) What type of cell(s) are affected by sex hormones? 3) Do sex hormones bring about cellular or subcellular changes in lymphocytes or in other cells which in turn influence the immune system?

Target Organs of Sex Hormone Action

Lymphatic Organs

Much of the earlier studies suggested that the thymus is the primary organ through which sex hormones bring about their action. First, the relationship between the thymus and gonads has been well documented.[173-178] For example, administration of gonadal hormones to animals has resulted in atrophy of the thymus.[99,175,176] Orchiectomy, particularly when performed in immature animals, has brought about a delay in thymic involution and thymic hyperplasia.[94,175,176] The hyperplasia of the thymus following orchiectomy is believed to be due to quantitative but not qualitative changes in thymocyte populations. There is an increase in cortisone-sensitive cells.[13] Interestingly, prepubertal orchiectomy of normal mice (Balb/c × SJL/J) resulted in an equal expansion of all thymocyte subsets.[179] In contrast, orchiectomy in autoimmune mice (NZB × SJL/5) led to selective expansion of dull Thy-1.2, Lyt-1, and dull PNA$^+$ cells, which thus suggested differential effects of sex hormones in health and disease. Thymic hormones, which greatly influence immune responses, are in turn affected by sex hormones. Estrogen reduces, and testosterone enhances, the levels of these hormones, but no significant effect of progesterone has been noted.[177] Conversely, the thymus also influences gonadal function. For example, neonatal thymectomy of mice has resulted in ovarian dysgenesis.[180,181] Furthermore, thymic hormones decrease sex hormones like progesterone by regulating the release of luteinizing hormone (LH) from the pituitary,[182-184] thus demonstrating the interrelationship of the thymus and gonads. Simultaneous gonadectomy and orchiectomy have abolished the immunopotentiation observed after orchiectomy alone.[6,94] Similarly, it was found that in B/W mice the thymus had to be present for demonstration of some of the effects of gonadectomy.[185] Based on the above findings, it was postulated that the thymus was the primary organ through which sex hormones brought about their action.[6,94] This view was further strengthened by the demonstration of SHRs in the thymus glands of both human and animals.[186,187] These receptors appear to be present in the thymic epithelial/reticular tissues but are more difficult to demonstrate on thymocytes. However, this may be due to very low capacity of these receptors and the use of whole lymphocyte populations, rather than particular subsets of lymphocytes which are sensitive to sex-hormonal action.[99]

However, other studies have shown that sex hormone effects can also be demonstrated in the absence of the thymus. Examples include the following: 1) sex hormone manipulation of thymectomized (and irradiated) rats profoundly altered autoimmune thyroiditis[68,122,125]; 2) administration of diethylstilbestrol to neonatally thymectomized mice reduced the numbers of T (Ly-1) cells in the spleen and the delayed type hypersensitivity reaction.[188]

Interestingly, SHRs have been found in the reticular tissues of lymph nodes and spleen suggesting that these are also the sites of sex hormone action.[189,190] In corroboration with this view, we noted profound effects of sex hormones on peripheral lymphoid organs of normal and SLE-prone autoimmune mice, providing presumptive evidence for sex hormone action on several lymphoid organs.[99] Furthermore, orchiectomy is also known to result in hyperplasia of the spleen and lymph nodes.[13,94,176]

In birds, the bursa is the primary target for sex hormone action. SHRs have also been demonstrated in this organ. Incubation of fertilized avian eggs in testosterone solution prevents the development of the bursa (agenesis) and atrophy of the cortical thymus. These birds have depressed responses to a variety of antigens at various times after maturity.

Thus, it appears that in addition to the thymus, other lymphoid organs may be important target sites for sex hormones (Figure 2).

The mechanisms of sex hormones at the cellular and subcellular (lymphocyte) level are much less clearly understood.

T Cells

Much of the recent evidence indicates that T cells are the primary target for sex hormone action (Figure 3). Examples include the profound effects of sex-hormonal manipulations on the thymocytes.[94,99,179,191] Further, sex hormones markedly modulate T-cell–mediated delayed-type hypersensitivity reactions.[17,94,129] Endocrine changes such as those in pregnancy have been associated with decreased T-cell numbers (OKT3$^+$ and OKT4$^+$) or T-cell function.[192,193] Sex steroids suppressed the lymphocyte proliferation in cultures stimulated by T-cell mitogen or antigen (Con A and phytohemagglutin [PHA]) PPD.[131,134,135]

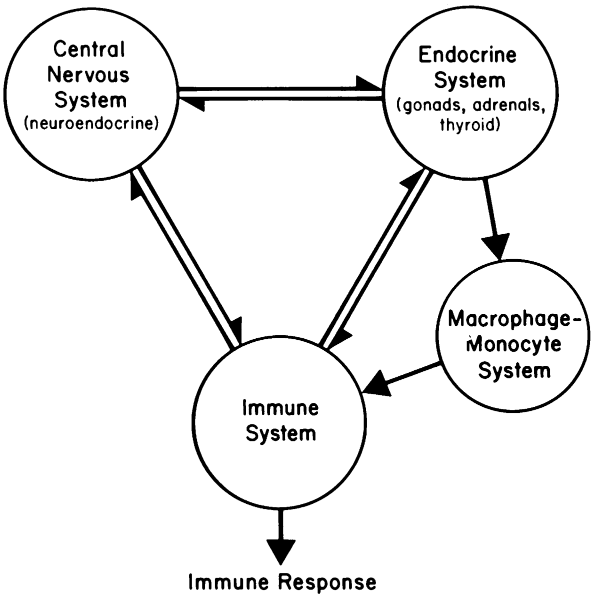


Figure 2—Immune responses are influenced by multiple systems. This illustration shows the influence of several systems on the immune system. There is a bidirectional influence between the immune and CNS and immune and endocrine system (particularly gonadal) and endocrine (gonadal) and CNS. Sex hormones act on the CNS, macrophage/monocyte system, or the immune system itself, to affect immune responses.

The effect of testosterone on T but not B cells has also been indicated in Tx-X rats with autoimmune thyroiditis, which is believed to be a T-cell–mediated disease. Testosterone treatment of Tx-X rats that were histopathologically and serologically positive brought about complete regression of the lesions but did not alter the levels of autoantibodies to thyroglobulin.[123]

Moreover, we have shown that short-term (2-week) administration of sex steroids to normal mice quantitatively altered Thy-1.2–positive cells in the thymus.[99] Long-term (4-month), but not short-term, treatment brought about an appreciable decrease in the numbers of Thyl-1.2–positive cells in peripheral lymphoid organs[120] (M.J. Dauphinee, S. Ansar Ahmed, N. Talal, manuscript in preparation). Similarly, bone marrow T cells bearing 20-α-hydroxysteroid dehydrogenase enzyme of B/W mice are sensitive to sex hormone manipulations.[119]

T cells stained with Lyt-2 monoclonal antibody were most sensitive to sex hormone action, particularly in the thymus.[99,120,191] In general, estrogen reduced, while androgens maintained, Lyt-2–positive cells.[99,191] There was a shift to the lower density in Lyt-2 antigens in androgen-treated mice, while the bright staining peak was either reduced or unaltered.[99] (M.J. Dauphinee, S. Ansar Ahmed, N. Talal, manuscript in preparation). Furthermore, studies in humans revealed that estrogen receptors are present on OKT-8–positive cells which have suppressor/cytotoxic function[194] and thymomas.[195] Estrogens suppress or deplete the functional activity of suppressor T cells, demonstrated by *in vitro* systems, both in humans[196] and in mice.[99] In contrast, male sex hormones maintain these activities. The inhibition of ^{3}H-thymidine uptake in PHA-stimulated cultures by syngeneic (suppressor) cells from sex-hormone–treated mice revealed a lack of definite correlation between Lyt-2–positive cells and the suppressor cellular activity. This suggests that sex hormones may also affect suppressor lymphocytes of as yet unknown phenotype. In any event, the modulation of suppressor cellular activity may explain, in part, the sex-differential immune responses.

Yet another mechanism of sex hormonal action is the modulation of T lymphokines (immunoregulatory hormones), interleukin-2 (IL-2), and γ-interferon (γ-IF). IL-2 is secreted by Lyt-1^+2^- T helper/inducer cell and acts on several important immunoregulatory cells which bear IL-2 receptors. All SLE-prone autoimmune mice have depleted IL-2 activity.[197] We observed that sex hormones modulate IL-2 activity; for example, androgens maintain this activity.[99,198] It is not known precisely how sex hormones affect IL-2 production. Nonetheless, manipulation of IL-2 levels by sex hormones can profoundly alter immunocapability.

Gamma-interferon, which is produced by lymphocytes (presumably T cells) after interaction with macrophages, also affects both cellular and humoral immunity. Sex hormones may modulate this lymphokine and thus affect the outcome of immune responses. However, as yet there is no evidence in this regard.

B Cells (Plasma Cells)

Sex hormones in many systems considerably alter antibody production or its levels (reviewed by Cohn[7]). This suggests that B cells are also targets for sex hormone action. In the context of autoimmunity, delayed testosterone treatment of B/W mice with established disease reduced autoantibodies to nucleic acids and prolonged survival times.[120,121] However, the reduction of these autoantibodies was not always a constant feature. It is possible that testosterone interfered with T helper cells or other T-cell signals necessary for autoantibody production rather than acting on B cells directly. A similar inference may be drawn in testosterone therapy studies in Tx-X rats with established thyroid autoimmunity in which testosterone failed to diminish the levels of these autoantibodies despite the regression of lesions.

We have recently noticed that there was no significant difference in lipopolysaccharide-induced proliferation of lymphocytes which were obtained from several normal strains of mice with differing sex hormonal environment. This again suggests that B cells may not be

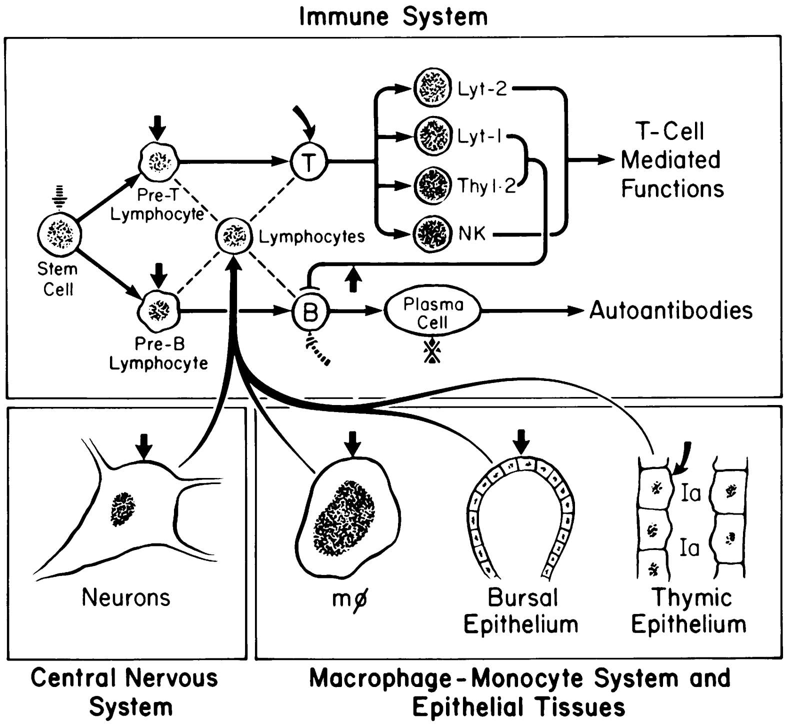


Figure 3—Sex steroid influence on lymphocytes. This illustration shows the major target cells of sex-hormone action (*arrows*). Sex hormones act on stem cells, thus affecting differentiation, maturation, and emigration of cells. Sex hormones also act on pre-T and pre-B cells, as well as mature T cells. In particular, Thy-1.2, Lyt-2, and Lyt-1 cells (and NK cells) are sensitive to sex hormones. These cells may be quantitatively or qualitatively (antigen density or function of cell) altered, which consequently influences immune responses. The major non-lymphoid target cells for sex-hormone action include neurons and neuroendocrine cells, fixed and free macrophages and monocytes, and thymic (and bursal) epithelial cells. Thus, the type of immune response is dependent upon the target cell for sex hormones and possibly the extent of sex-hormonal action. *Interrupted arrows* indicate that the action of sex hormone on these cells is not definitively established.

the targets for sex hormone action. Furthermore, sex hormones given to mice did not greatly alter the numbers of $F(ab)_2$-positive cells (B cells). However, in sex-hormone–treated *lpr* mice, there were increased numbers of B cells in the thymus.[99] This may be a compensatory increase following depletion of T cells. Moreover, studies on pokeweed-induced immunoglobulin synthesis by human peripheral blood lymphocytes containing estradiol revealed that estrogen does not act directly on B cells. Furthermore, SHRs on B cells have not yet been demonstrated.

Nonetheless, sex hormones may affect certain other B-dependent antigens. Furthermore, it is likely that sex hormones may selectively influence certain subpopulation(s) of B cells such as the Ly-1$^+$B positive cell. This cell is known to produce a variety of autoantibodies, including to bromelin-treated mouse red blood cells (Br-MRBCs). We are currently examining the precise effects of sex hormones on B cells in normal and autoimmune mice.

In light of available evidence, overall it appears that sex hormones may indirectly (or directly) affect the initial stages of antibody production by B cells, but do not affect plasma cells (Figure 3).

Natural Killer (NK) Cells

The importance of NK cells is of current immunobiologic interest.[199,200] These cells play an important part in spontaneous cytotoxicity for a variety of viral-infected or malignant cells. However, the role of NK cells in autoimmune diseases is as yet uncertain. Nevertheless, studies in this laboratory have shown that SLE-prone female mice bearing the *lpr* gene have depleted splenic NK cell activity (Z. Li-Phan, M. J. Dauphinee, S. Ansar Ahmed, N. Talal, manuscript in preparation). Sex hormones, particularly estrogen (natural and synthetic), markedly reduce NK cell activity in B/W and normal mice (Figure 3).[201-203] The precise mechanism of depletion of this cellular activity by estrogen is not readily apparent. One such mechanism may be reduced influx of these cells from bone marrow which becomes obliterated due to osteoproliferation. Alternatively, this may be an indirect consequence of alteration of IL-2 and γ-IF levels, which are known to influence the NK cellular activity. Yet another possibility is the generation of suppressor cells for NK-cell activity. These aspects are currently under investigation in our laboratory.

Thymic Epithelium/Bursal Epithelium

Nonlymphoid cells are also targets for sex hormone action (Figure 3). Sex hormone receptors have been found on thymic epithelium and reticular tissues and profoundly influence lymphocyte morphology and function. One way by which sex hormones may act is by influencing the thymic microenvironment and thymic hormones and thereby greatly affecting T-cell differentiation, maturation, and migration. Furthermore, sex hormonal action on these cells may also influence Ia-antigen expression, which is important in many aspects of lymphocyte biology. Thymic hormonal changes induced by sex hormones may act on the hypothalamus to release gonadotropic hormones which exert profound immunoregulatory effects.

Sex hormones may also indirectly influence B-cell maturation and differentiation. For example, studies on transplantation of bursa into testosterone-treated birds revealed that the hormones affects the epithelial cells but not the lymphoid components of the bursa,[204-205] thereby indirectly influencing B-cell function.

Macrophage Monocyte System

Sex hormones also act on macrophages/monocytes, which are important for autoantigen processing and presentation in immunogenic form to lymphocytes (Figures 2 and 3). For example, estrogen greatly increased the ability of these cells to phagocytose particulate antigen.[206] Estrogens stimulate the reticuloendothelial system, division of Kupffer cells in the liver,[207,208] the numbers of circulating monocytes,[207,209] and peritoneal macrophages.[209,210] Sex hormones modulate the clearance of mouse erythrocyte-IgG complexes in B/W mice. Estrogen-treated mice had delayed clearance, while testosterone augmented the clearance of these immune complexes. The discrepancy over the effects of estrogens on reticuloendothelial cells may be due to obvious differences in the experimental situation; for example, estrogen may have different effects in normal and autoimmune mice or with different doses and routes of administration. Nevertheless, these results do indicate the influence of sex hormones on this system. Indeed, SHRs have also been found on the reticular tissues of thymus, lymph nodes, spleen, and arterial epithelium.[189]

Central Nervous System (CNS)

Sex hormones can also influence the immune system via acting on the CNS (Figure 2). SHRs have been found at several sites in the brain. These aspects have been discussed in greater detail elsewhere.[211,212] It has now become apparent that certain areas of the CNS actively collaborate with the immune system in affecting immune responses.[211,212] Of particular interest are the neurons of the ventromedial nucleus (VMN) of the hypothalamus, which modify immune responses. Interestingly, the VMN of hypothalamus is enriched in SHRs. Thus, it seems plausible that sex hormones may bind to neurons of the VMN (and preoptic nucleus) and generate activities which influence the immune system.

Osteal Tissues

Sex hormones may also affect the immune system by causing changes in tissues which are in close apposition to lymphoid tissues. For example, estrogen causes endosteal proliferation, resulting in the occlusion of bone marrow. Recent studies have demonstrated SHRs in endosteal tissues (P. Sheridan, personal communication). High doses result in complete replacement of bone marrow and thus interfere with the emigration of cells to the thymus and other peripheral lymphoid organs. For example, NK-cell activity in the spleen, which is believed to depend on the bone marrow, is markedly reduced after estrogen administration.

Other Endocrine Organs

Sex hormones may act on other endocrine or neuroendocrine organs to bring about their effects (Figure 2). For example, sex hormones may influence the pituitary hormonal production, which in turn stimulates several endocrine glands to release their products, which modulate immune responses. However, some experimental situations employing combined adrenalectomy and ovariectomy have shown that the immunosuppression mediated by estrogen is not mediated through adrenal or ovarian function.[213] Recently, SHRs have been found in thyroids of primates (P. Sheridan, personal communication). Thus, sex hormones may bind to these cells to influence the release of thyroxine, which in turn influences immune responses.

Other Nonlymphoid Tissues

Enzymatically Active Cells

Similarly, estrogens have been shown to decrease the activity of the enzyme NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase (PGDH)[214] in kidney tissue, whereas androgens increase it.[215] The PGDH-specific activity in the kidney tissue of male and female B/W F_1 mice was tested and found to be higher in males.[215] The specific activity of PGDH was found to be inversely related to the severity of the autoimmune disease. It remains to be determined whether the alteration of this enzyme activity is a direct effect of sex hormones or an epiphenomenon. Furthermore, the specific role of this enzyme in the pathogenesis of the disease is not known. An enzyme, 20-α-hydroxysteroid dehydrogenase (20-αSDH), on T cells is also affected by testosterone. Castration decreased 20-α-SDH positive cells, whereas testosterone administration resulted in its increase.[119]

Complement-Producing Cells

Sex hormones may act on complement-producing cells and influence the synthesis of complement components, particularly C4 and C5. Coincidentally, genes controlling various facets of testosterone physiology (such as rate of production and its binding capacity) and those determining complement production reside within or close to the H-2 complex. Thus, both sex hormones and complement may closely interact. Alterations of complement levels by sex hormones can markedly affect complement-dependent immune responses. It is of particular interest that danazol, weakly androgenic, increases the concentration of Cl esterase inhibitor in humans and is useful in the treatment of hereditary angioneurotic edema. It is not clearly known whether the beneficial effects of danazol in idiopathic thrombocytopenia with or without lupus, is due to a similar biochemical effects.

Cells Producing Secretory Components

Mucosal epithelial cells, lacrimal glandular cells and hepatocytes which produce secretory components are important in local (secretory) IgA-dependent immunity.[216] These cells may also be the direct or indirect targets for sex hormone action.[217,218] For example, orchiectomy decreases free secretory components in tears of rats, while androgen administration reverses this effect.[218] Interestingly, secretory component production is androgen-dependent and is not influenced by female sex hormones.

Overall, the diverse target sites of sex hormone action through which immune responses are influenced is now apparent.

Sex Hormones and Immune Imbalance

The concept of a balance between humoral and cellular immunity that maintains the normal state was suggested a long time ago.[2] An equilibrium between regulatory cells may be important for the maintenance of immune homeostasis and prevention of immunologic abnormalities such as autoimmune diseases. Thus, an imbalance between lymphocyte subpopulations, rather than an absolute deficiency of certain types of cells, may lead to disease. Sex hormones may critically alter this balance and the tendency to autoimmunity. Two lines of evidence support this proposition: 1) First, lymphoid cells obtained from rats of different sex hormonal status were transferred to Tx-X female recipient, and the effect on the development of thyroid autoimmunity in the recipients was used as an *in vivo* index (Ansar Ahmed and Penhale, manuscript in preparation). Such studies revealed that sex hormones bring about functional alterations between the helper (effector) and suppressor cells. 2) Direct evidence for the alteration of suppressor (Lyt-2)/helper (Ly-1) subsets in normal or autoimmune mice by sex hormones has now been reported.[99,191]

Concept of Qualitative Alterations of Lymphocytes by Sex Hormones

Sex hormones may influence the qualitative functions of lymphocytes. It has been shown that steroid hormones like glucocorticosteroids act directly on the DNA of the cell and modify the transcription of specific genes.[219] Similarly, sex steroids may directly act on the DNA of the lymphocytes and modulate its function (eg, modulate mRNA synthesis) or bring about alterations in the expression of surface antigens, thereby interfering with cellular interactions. Autoradiography studies have revealed that ^{3}H-testosterone is incorporated into the nucleus of human lymphocytes.[220] Sex hormones may alter the microenvironment to induce migratory or trafficking changes in lymphocytes. These aspects will be fully clarified with further technical advances in the future.

Postulated Mechanisms of Sex Hormone Action in B/W Mice and Tx-X Rats

As discussed earlier, the mechanisms of sex hormone action in B/W mice and Tx-X rats are extremely complex. Thymus appears to be the major site of action in B/W mice. Sex hormones act on the thymus and bring about quantitative and qualitative changes in thymocyte subpopulations. These may be a consequence of a direct action on thymocytes or the result of affecting the thymic microenvironment by altering the release of thymic hormones. Additionally, thymic hormones affect the release of pituitary hormones, which have a profound effect on the immune systems. Not all cells appear to be sensitive to sex hormone action. Immunoregulatory cells bearing Thy-1.2, Lyt-2, or antigenic markers (Ly-1) appear to be more sensitive to sex hormonal action. Furthermore, sex hormones may act on the Ia-positive thymic epithelium which has important role in directing lymphocyte specificity and interactions with other lymphocytes. Thus, sex hormones can act on the thymus and affect their function and maturation, and emigration of cells to the periphery.

Hormones, like estrogen, may additionally affect the influx of cells from the bone marrow to the thymus.

In the Tx-x model, bone marrow may be an important site for sex hormone action, because these animals are deprived of their thymus. Other lymphoid organs and nonlymphoid organs are also a site for sex hormone action and may act in concert to affect autoimmune diseases. Akin to the murine model, transfer studies have revealed that sex hormones alter the balance of helper to suppressor cells.

Conclusions

Studies on the relationship of sex hormones with the immune system are important for three reasons: 1) Sex hormones influence the normal differentiation, maturation, and emigration of lymphocytes, which is vitally important in terms of immune capabilities. 2) Naturally occurring sex hormones modulate the course of events of autoimmune diseases. Thus, sex hormones may be used as an endogenous tool in the study of pathologic events of autoimmune diseases. A correct endocrine balance may be crucial for immune homeostasis, ie, prevention or induction of autoimmune diseases. 3) Immunosuppressive male sex hormones may be advantageously exploited clinically in the treatment of immunologic hyperreactivity disorders. In particular, a combination of danazol and anti-estrogen drugs may prove to be of therapeutic benefit.

The effects of sex hormones on immunity should be seen in a broader context that we have termed immunoendocrinology, which encompasses the bidirectional interactions between three major systems: the classic endocrine system, the neuroendocrine system, and the immune system. The influence of sex factors (sex hormones) on autoimmune diseases is undisputed. Studies from several laboratories indicate that sex hormones act on multiple systems (Figure 2) and multiple cells (Figure 3). Thus, the type of sex-hormone–influenced immune response, immunosuppression or immunoenhancement, is dependent upon where sex hormones act. In any event, the ultimate responder cell is a lymphocyte. Sex hormones may alter the function of lymphocytes, thereby affecting production of immunoregulatory factors, expression of cell surface antigens, or ability to interact with other cells. Available evidence indicates that T cells may be major targets for sex-hormone action. Sex hormones may quantitatively or qualitatively influence the levels of immunoregulatory cells, such as suppressor and helper cells. Due to the complexity or diversity of suppressor T cells in several systems, the precise effects of sex hormones on suppressor cells cannot be conclusively determined. It is hoped, with further understanding in the fields of immunoendocrinology and molecular biology and advancements in technology, the effects of sex hormones on lymphocytes will become fully clarified.

References

1. Lambert PH, Dixon FJ: Pathogenesis of the glomerulonephritis of NZB/W mice. J Exp Med 1968, 127: 507–522
2. Talal N: Disordered immunologic regulation and autoimmunity. Transplant Rev 1976, 31:240–258
3. Penhale WJ, Farmer A, McKenna RP, Irvine WJ: Spontaneous thyroiditis in thymectomized and irradiated wistar rats. Clin Exp Immunol 1973, 15:225–236
4. Penhale WJ, Ansar Ahmed S: Autoimmune thyroiditis, Model 248, Animal Models of Human Disease. Fascicle 11. Edited by CC Capen, DB Hackel, TC Jones, G Migaki. Registry of Comparative Pathology, Armed Forces Institute of Pathology, Washington, DC, 1982
5. Batchelor JR: Hormonal control of antibody formation, Regulation of the Antibody Response. Edited by B Cinader. Springfield, Charles C. Thomas, 1968, pp 276–293
6. Sijivic VS, Warr GW: Activity of the reticuloendothelial system and the antibody response: II. Effect of stilbesterol on the immune response to sheep erythrocytes in the mouse. Br J Exp Pathol 1973, 54:69–78
7. Cohn DA: High sensitivity to androgen as a contributing factor in sex differences in the immune response. Arthritis Rheum 1979, 22:1218–1233
8. Butterworth M, McClellan B, Allansmith M: Influence of sex on immunoglobulin levels. Nature 1967, 214: 1224–1225
9. Rowe DS, McGreggor IA, Smith SJ, Hall P, Williams K: Plasma immunoglobulin concentrations in a West African (Gambian) community and in a group of healthy British adults. Clin Exp Immunol 1968, 3:63–79
10. Buckley CE III, Dorsey FC: Serum immunoglobulin levels throughout the life-span of healthy man. Ann Intern Med 1971, 75:673–682
11. Licthman MA, Vaughan JH, Hames CG: The distribution of serum immunoglobulins, anti G globulins ("rheumatoid factor") and antinuclear antibodies in white and negro subjects in Evans County, Georgia. Arthritis Rheum 1967, 10:204–215
12. Rhodes K, Markham RL, Maxwell PM, Monk-Jones NE: Immunoglobulins and the X-chromosome. Br Med J 1969, 3:439–441
13. Eidinger D, Garrett TJ: Studies of the regulatory effects of the sex hormones on antibody formation and stem cell differentiation. J Exp Med 1972, 136:1098–1116
14. Stern K, Davidsohn I: Effect of estrogen and cortisone on immune hemoantibodies in mice of inbred strains. J Immunol 1955, 74:479–484
15. Terres G, Morrison SL, Habicht GS: A quantitative difference in the immune response between male and female mice. Proc Exp Biol Med 1968, 127:664–667
16. Brent L, Medawar P: Quantitative studies on tissue transplantation immunity: VII. The effect of irradiation. Proc R Soc London [Biol] 1966, 165:413–424
17. Graff RJ, Lappe MA, Snell GD: The influence of the gonads and adrenal glands on the immune response to skin grafts. Transplantation 1969, 7:105–111
18. Kongshavn PAL, Bliss JQ: Sex differences in survival of H-2 incompatible skin grafts in mice treated with antithymocyte serum. Nature (Lond) 1969, 226:451
19. Orsini MW, Blazkovec AA: Sexual dimorphism in the

secondary immune response of the syrian hamster. Int Arch Allergy Appl Immunol 1974, 46:858
20. Santoli D, Trinchieri G, Zinijewski CM, Koprowski, H.: HLA related control of spontaneous and antibody dependent cell mediated cytotoxic activity in humans. J Immunol 1976, 117:765-770
21. Barnes EW, MacCuish AC, London AC, Jordan J, Irvine IJ: Phytohaemagglutinin-induced lymphocyte transformation and circulating autoantibodies in women taking oral contraceptives. Lancet 1974, I:898-900
22. Weinstein Y, Ran S, Segal S: Sex associated differences in the regulation of immune responses controlled by the MHC of the mouse. J Immunol 1984, 132:656-661
23. Dresser DW: Specific inhibition of antibody production. I. Protein-over-loading paralysis. Immunology 1962, 5:161-168
24. Gross L: Influence of sex on the evolution of a transplant mouse sarcoma. Proc Soc Exp Biol Med 1941, 47:273-276
25. Snell KC: In *The Pathology of Laboratory Animals.* Edited by WE Ribelin Jr, McCoy JR. Charles C Thomas, Springfield (1965) p. 241
26. Meissner WA, Diamdopoulos GTH: Neoplasia. In *Pathology* Vol. I, Edited by WAD Anderson and JM Kissane. The CV Mosby Company, St. Louis (1977) p. 674
27. Mobius P: Geschlect und Krankheit. Beitr. Lehre. Chlects. Unterschieden. Verlag Carl Marhold. Hall a.d.s. many (1903) p. 39
28. Lenz F: Morbific heriditary factors. In *Human Hereditary.* Edited by E Baur, E Fisher and F Lenz. Macmillan, New York, (1931) p. 37.
29. Willoughby DS, Watson DW: Host-parasite relationships among group A streptococci II. Influence of sex on susceptibility of inbred mice towards streptococcal infection. J Bact 1969, 87:1457-1461
30. Berkovich S, Ressel M: Effect of sex on susceptiblity of adult mice to Coxsackie B virus infection. Arch Gesamte Virus Forsch 1967, 22:246-251
31. Goble FC, Konopka EA: Sex as a factor in infectious disease. Trans NY Acad Sci 1971, 35:325-346
32. Rifkind D: Influence of gonadectomy on *Candida albicans* urinary tract infection in CFW mice. Infect Immun 1972, 5:363-369
33. Rifkind D, Frey J: Sex differences in antibody response of CFW mice to *Candida albicans.* Infect Immun 1972, 5:695-698
34. Andersen AA, Hanson RP: Influence of sex and age on natural resistance to St. Louis encephalitis virus infection in mice. Infect Immun 1974, 9:1123-1125
35. Salomon CB: Host hormones and parasitic infection, International Review of Tropical Medicine. Edited by DR Lincome, AW Woodruff. New York, Academic Press, 1969, pp 101-158
36. Noble ER, Noble GA: Parasite-host responses, Parasitology, the Biology of Animal Parasites. Edited by ER Noble, GA Noble. Philadelphia, Lea and Febiger, 1976, pp 426-434
37. Huber SA, Job PL, Auld JK, Woodruff JF: Sex related differences in the rapid production of cytotoxic spleen cells against uninfected myofibers during coxsackievirus-B infection. J Immunol 1981, 126:1336-1340
38. Pease M: How long do poultry breeding stock live? J Ministry Agric 1947, 54:263-268
39. Vital Statistics of the US. 1972. Vol II. Mortality, Part A. US Department of Health, Education and Welfare, Rockville, Maryland, 1976
40. Beverton RJH, Holt SJ: A review of the lifespans and mortality rates of fish in nature and their relation to growth and other physiological characteristics, Ciba Foundation Colloquia on Aging. Vol 5. The Life Span of Animals. Edited by GEW Wolsteinhome, M O'Connor. Boston, Mass, Little Brown, 1959, pp 142-148
41. Simms HS: Longevity studies in rats: I. Relation between life span and age of onset of specific lesions, Pathology of Laboratory Rats and Mice. Edited by E Cotchin, FJC Roe. Philadelphia, F. A. Davis, 1967, p 773
42. Hamilton HB, Hamilton RS, Mestler GE: Duration of life and cause of death in domestic cats: Influence of sex, gonadectomy and inbreeding. J Gerontol 1969, 24:427-437
43. Williams ED, Doniach I: Postmortem incidence of thyroiditis. J Pathol Bacteriol 1962, 83:255-264
44. Doniach D, Roitt IM: Autoimmune thyroid disease, Textbook of Immunopathology. Vol II. Edited by PA Miescher, HJ Muller-Eberhard. New York, Grune and Stratton, 1976, p 715
45. Kahn CR, Flier, JS: Immunologic aspect of endocrine disease, Clinical Immunology. Edited by CW Parker. Philadelphia, W. B. Saunders, 1980, p 815
46. Rallison ML, Dobyns BM, Keating FR, Rall JE, Taylor FH: Occurrence and natural history of chronic lymphocytic thyroiditis in childhood. J Pediat 1975, 86:675-682
47. Steihm ER, Fulginiti VA: Immunologic aspects of pediatric illness, Immunologic Disorders in Infants and Children. Edited by ER Steihm, VA Fulginiti. Philadelphia, W. B. Saunders 1980, p 584
48. Dubois EL: Lupus erythematosus, Lupus Erythematosus. Edited by EL Dubois. Los Angeles, University of Southern California Press, 1974, p 340
49. Stern R, Fishman J, Brushman H, Kunkel HG: Systemic lupus erythematosus associated with Klinefelter's syndrome. Arthritis Rheum 1977, 20:18-22
50. Inman RD: Immunologic sex differences and the female predominance in systemic lupus erythematosus. Arthritis Rheum 1978, 21:849-852
51. Glynn LE, Holborrw EJ (Eds) Rheumatoid arthritis, Autoimmunity and Disease. Oxford, Blackwell, 1965, p 132
52. Christian CL. Rheumatoid arthritis, Cecil Textbook of Medicine. Edited by JB Wyngarden, LH Smith. Philadelphia, W. B. Saunders, 1982, p 1845
53. Whaley K, Buchanan WW: Sjogrens syndrome and associated diseases, Clinical Immunology. Edited by CW Parker. Philadelphia, W. B. Saunders, 1980, p 632
54. Rose NR: Endocrine diseases, Basic and Clinical Immunology. Edited by HH Fudenberg, OP Stites, JL Caldwell, JV Wells. Los Altos, Lange Medical Publications, 1980, p 644
55. Harris ED Jr: Scleroderma, Cecil Textbook of Medicine. Edited by JB Wyngarden, LH Smith. Philadelphia, W. B. Saunders 1982, p 1857
56. Rodnan GP: Progressive systemic sclerosis (scleroderma), Clinical Immunology. Edited by CW Parker. Philadelphia, W. B. Saunders 1980, p 784
57. Schwab RS, Leland CC: Sex and age in myasthenia gravis as critical factors in incidence and remission. JAMA 1953, 153:1270-1273
58. Cook RD: Multiple sclerosis: is the domestic cat involved? Medical Hypothesis 1981, 7:147-154
59. Chapel H, Haeney M (eds): Essentials of Clinical Immunology. Oxford, Blackwell Scientific Publications, 1984, p 267
60. Couchman KG, Wigley RD, Prior IAM: Autoantibodies in the Carterton population survey. J Chron Dis 1970, 23:45-53
61. Khangure MS, Dingle PR, Stephenson J, Bird T, Hall R, Evered DC: A long term follow up of patients with autoimmune thyroid disease. Clin Endocrinol 1977, 6:41-48
62. Rose NR, Bacon LD, Sundick RS, Kong YM, Esquivel

PS, Bigazzi P: Genetic regulation in autoimmune thyroiditis, Autoimmunity: Genetic, Immunologic and Clinical Aspects. Edited by N Talal. NY, Academic Press, 1977, p 63
63. Bacon LD, Kite JH Jr, Rose NR: Relation between the major histocompatibility (B) locus and autoimmune thyroiditis in obese chickens. Science 1974, 186:274-276
64. Lillehoj H, Beisel K, Rose NR: Genetic control of experimental autoimmune thyroiditis in rats. J Immunol 1981, 127:654-660
65. Penhale WJ, Farmer A, Urbanaik SJ, Irvine WJ: Susceptibility of inbred rat strains to experimental thyroiditis: Quantitation of thyroglobulin binding cells and assessment of T cell function in susceptible and nonsusceptible strains. Clin Exp Immunol 1975, 19:179-191
66. Reuber MD, Glover EL: Role of age and sex in chronic thyroiditis in rats fed 3-methyl-4-dimethyl-aminoazobenzene. Vet Pathol 1976, 13:295
67. Ansar Ahmed S, Penhale WJ: The role of sex steroids in autoimmune thyroiditis. Fourth International Congress of Immunology. Paris Abstract. Edited by JL Preud'Homme and VAL Hawken. Paris, Academic Press, 1980, p 14.3.02
68. Penhale WJ, Ansar Ahmed S: The effect of gonadectomy on the sex-related expression of autoimmune thyroiditis in thymectomized and irradiated rats. Am J Reprod Immunol 1981, 1:326-330
69. Kojima A, Tanaka-Kojima Y, Sakakaurar R, Nishizuka Y: Sponaneous development of autoimmune thyroiditis in neonatally thymectomized mice. Lab Invest 1976, 34:550-558
70. Steinberg AD, Melez KA, Raveche ES, Reeves JP, Boegel WA, Smathers PA, Taurog JD, Weinlein L, Duvic M: Approach to the study of the role of sex hormone in autoimmunity. Arthritis Rheum 1979, 22:1170-1176
71. Morton JI, Weyant DA, Siegel BV, Golding B: Androgen sensitivity and autoimmune disease. Immunology 1981, 44:661-668
72. Howie JB, Holyer JB: The immunology and pathology of NZB mice. Adv Immunol 1968, 9:215-266
73. Roubinian JR, Talal N, Greenspan JS, Goodman JR, Siiteri K: Effect of castration and sex hormone treatment survival, anti-nucleic acid antibodies and glomerulonephritis in NZB x NZW F1 mice. J Exp Med 1978, 147:1568-1583
74. Raveche ES, Tijo JH, Steinberg AD: Genetic studies in NZB mice: IV. The effect of sex hormones on spontaneous anti-T cell antibodies. Arthritis Rheum 1980, 23:48-56
75. Murphy ED, Roths JB: Autoimmunity and lymphoproliferation: Induction by mutant gene lpr and acceleration by a male-associated factor in strain BXSB mice, Genetic Control of Autoimmune Disease. Edited by N Rose, PE Bigazzi, NL Warner. New York, Elsevier/North Holland, 1978, pp. 207-221
76. Hang LM, Balderas R, Fieser T, Dixon FJ, Theofilopoulos AN: Effects of accelerating factor (AF) on autoimmune (AI) manifestations of mice with different backgrounds (Abstr). Fed Proc 1984, 43:1833
77. Lewis RM: Systemic lupus erythematosus in dogs, The Canine as a Biomedical Research Model: Immunological, Hematological, and Oncological Aspects. Edited by M Shifrine, FD Wilson. Technical Information Center, U.S. Dept of Energy, Vermont, Virginia 1980
78. Allen JB, Blatter D, Calandrea GB, Wilder RL: Sex hormonal effects on the severity of streptococcal cell with induced polyartheritis in the rat. Arthritis Rheum 1983, 26:560-565
79. Bielschowsky M, Helyer BJ, Howie JB: Spontaneous haemolytic anaemia in mice of the NZB/BL strain. Proc Univ Otag Med Sch 1959, 37:9-13
80. Milich DR, Gershwin ME: Murine autoimmune hemolytic anemia via xenogeneic erythrocyte immunization: III. Differences of sex. Clin Immunol Immunopathol 1981, 18:1-11
81. Wilkins RJ: Autoimmune thrombocytopenia in dogs, Current Veterinary Therapy. Vol VI. Edited by RW Kirk. Philadelphia, W. B. Saunders, 1977, p 445
82. Clark HC, Childress RO, Coleman NC: Idiopathic thrombocytopenic purpura: A review and case report. Vet Med Sm Ani Clin 1980, 75:427-430
83. Roubinian JR, Papoian R, Talal N: Androgenic hormones modulate autoantibody responses and improve survival in murine lupus. J Clin Invest 1977, 59:1066-1070
84. Schot LPC, Verheul AM, Schuurs AHWM: Effect of nondrolone decanoate on Sjogren's syndrome like disorders in NZB/NZW mice. Clin Exp Immunol 1984, 57:571-574
85. Polley CR, Bacon LD, Rose NR: Spontaneous autoimmune thyroiditis in chickens. J Immunol 1981, 127: 1465-1468
86. Murphy ED, Roths JB: A Y chromosome-associated factor in strain BXSB producing accelerated autoimmunity and lymphoproliferation. Arthritis Rheum 1979, 22:1188-1193
87. Kitchen DN, Todd GC, Meyers DB, Paget C: Rat lymphocytic thyroiditis associated with ingestion of an immunosuppressive compound. Vet Pathol 1979, 16: 722-729
88. Simon DB, Ringel SP, Sufit RL: Clinical spectrum of fascial inflammation. Muscle and Nerve 1982, 5:525-537
89. Rhodes K, Scott A, Markham RL, Monk-Jones ME: Immunological sex differences: A study of patients with rheumatoid arthritis, their relatives, and controls. Ann Rheum Dis 1969, 28:104-120
90. Grundbacher FJ: Human X chromosome carries quantitative genes for immunoglobulin M. Science 1972, 176:311-312
91. Adinolfi M, Haddad SA, Seller MJ: X chromosome, complement and serum levels of IgM in man and mouse. Immunogenetics 1978, 5:149-156
92. Eisenberg RA, Dixon J: Effect of castration on male-determined acceleration of autoimmune disease in BXSB mice. J Immunol 1980, 125:1959-1963
93. Barnabei VM, Sakamoto K, Seeley JK, Purtiulo DT: Chromosomal breakage and sister chromatid exchange in peripheral blood lymphocytes and lymphoblastoid cell lines in the x-linked lymphoproliferative syndrome. Cancer Genet Cytogen 1982, 6:313-321
94. Castro JE: Orchidectomy and the immune response. II. Response of orchidectomized mice to antigens. Proc R Soc Lond [Biol] 1974, 185:437-451
95. Albin RJ, Bruns GR, Guinan P, Bush IM: The effect of estrogen on the incorporation of ^{3}H-thymidine by PHA-stimulated human peripheral blood lymphocytes. J Immunol 1974, 113:705-707
96. Fujii H, Nawa Y, Tsuchiya H, Matsuno K, Fukumoto T, Fukuda S, Kotani M: Effect of a single administration of testosterone on the immune response and lymphoid tissues in mice. Cell Immunol 1975, 20:315-326
97. Grossman CJ: Regulation of the immune system by sex steroids. Endocr Rev 1984, 5:435-454
98. Luster MI, Hayes HT, Korach K, Tucker AN, Dean JH, Greenlee WF, Boorman GA: Estrogen immunosuppression is regulated through estrogenic responses in the thymus. J Immunol 1984, 133:111-116
99. Ansar Ahmed S, Dauphinee MJ, Talal N: Effects of short term administration of sex hormones on normal and autoimmune mice. J Immunol 1985, 134:204-210
100. Lahita RG, Bradlow HL, Kunkel HG, Fishman J: Alterations of estrogen metabolism in systemic lupus erythematosus. Arthritis Rheum 1979, 22:1195-1198
101. Jungers P, Nahoul K, Pelissier C, Dougados M, Tron

F, Bach JF: Low plasma androgens in women with active or quiescent systemic lupus erythematosus. Arthritis Rheum 1982, 25:454–457
102. Smolen JS, Steinberg AD: Systemic lupus erythematosus and pregnancy. Clinical, immunological and theoretical aspects, Reproductive Immunology. Edited by N Gleicher. New York, Liss, 1981, p 283–302
103. Hayslett JP, Lynn RI: Effect of pregnancy in patients with lupus nephropathy. Kidney Int 1980, 18:207–220
104. Chapel TA, Burns RE: Oral contraceptives and exacerbations of lupus erythematosus. Am J Obstet Gynecol 1971, 110:366–369
105. Jungers P, Dougados M, Pelissier C, Nahoul K, Tron F, Bach J-F: Influence of oral contraceptive therapy on the activity of systemic lupus erythematosus. Arthritis Rheum 1982, 25:618–623
106. Amino N, Miyai K, Kuro R, Taniznawa O, Azukizawa M, Takai S, Tanaka F, Nishi K, Kahwashima M, Kumahara Y: Transient postpartum hypothyroidism: fourteen cases with autoimmune thyroiditis. Ann Int Med 1977, 87:155–159
107. Amino N, Miyai K, Yamamoto T: Transient recurrence of hyperthyroidism after delivery in Graves' disease. J Clin Endocrinol Metab 1977, 44:130–136
108. Hench PA: The potential reversibility of rheumatoid arthritis. Mayo Clin Proc 1949, 24:167–178
109. Lorz HM, Frumin AM: Spontaneous remission in chronic idiopathic thrombocytopenic purpura during pregnancy. Obstet Gynecol 1961, 17:362–363
110. Gutierrez G, Dagnino R, Mintz G: Polymyositis/dermatomyositis and pregnancy. Arthritis Rheum 1984, 27:291–294
111. Chopra IJ, Tulchnisky D: Status of estrogen-androgen balance in hyperthyroid men with Graves' disease. J Clin Endocrinol Metab 1974, 38:269–277
112. McKenna CH, Weiman KC, Schulman LE: Oral contraceptives, rheumatic diseases, and autoantibodies (Abstr). Arthritis Rheum 1969, 12:313–314
113. Schleicher EM: LE cells after oral contraceptives. Lancet 1968, 1:821–822
114. Spiera H, Plotz CM: Rheumatic symptoms and oral contaceptives. Lancet 1969, 1:571–572
115. Garovich M, Agudelo C, Pisko E: Oral contraceptives and systemic lupus erythematosus. Athritis Rheum 1980, 23:1396–1398
116. Gluud C, Tage-Jensen U, Bahnsen M, Dietrichson O, Svejgaard A: Autoantibodies histocompatibility antigens and testosterone in males with alcoholic cirrhosis. Clin Exp Immunol 1981, 44:31–38
117. Roubinian J, Talal N, Siiteri P, Sadakian JA: Sex hormone modulation of autoimmunity in NZB/NZW mice. Arthritis Rheum 1979, 22:1162–1169
118. Verheul HAM, Stimson WH, Hollander FCD, Schuurs AHWM: The effects of nondrolone, testosterone and their decanoate esters on murine lupus. Clin Exp Immunol 1981, 44:11–17
119. Weinstein Y, Berkovich Z: Testosterone effect on bone marrow, thymus and suppressor T cells in the (NZB X NZW)Fl mice: Its relevance to autoimmunity. J Immunol 1981, 128:998–1002
120. Talal N, Dauphinee MJ, Ansar Ahmed S, Christadoss P: Sex factors in immunity and autoimmunity, Progress in Immunology. Vol V. Edited by Y Yamamura, T Tada. New York, Academic Press, 1983, p 1589–1600
121. Roubinian JR, Talal N, Greenspan JS, Goodman JR, Siiteri PK: Delayed androgen treatment prolongs survival in murine lupus. J Clin Invest 1979, 63:902–911
122. Ansar Ahmed S, Penhale WJ: The influence of testosterone on the development of autoimmune thyroiditis in thymectomized and irradiated rats. Clin Exp Immunol 1982, 48:367–374
123. Ansar Ahmed S, Young PR, Penhale WJ: Beneficial effects of testosterone in the treatment of chronic autoimmune thyroiditis in rats. J Immunol (In press)
124. Ansar Ahmed S, Penhale WJ: Thyroid transplantation in rats developing autoimmune thyroiditis following thymectomy and irradiation. Clin Exp Immunol 1981, 45:480–486
125. Ansar Ahmed S, Young PR, Penhale WJ: The effects of female sex steroids on the development of autoimmune thyroiditis in thymectomized and irradiated rats. Clin Exp Immunol 1983, 54:351–358
126. Kittas C, Henery L: Effect of sex hormones on the immune system of guinea-pigs and on the development of toxoplasmic lesions in nonlymphoid organs. Clin Exp Immunol 1979, 36:16–23
127. Grossman CJ, Sholiton LJ, Roselle G: Estrodiol regulation of thymic lymphocyte function in the rat. J Steroid Biochem 1982, 16:683–690
128. Mueller AP, Wolfe HR, Meyer RK: Precipitin production in chickens. XXI. Antibody production in bursectomized chickens and in chicken injected with 19-nortestosterone on the fifth day of incubation. J Immunol 1960, 85:172–179
129. Frey-Wettstein, Craddock CA: Testosterone induced depletion of thymus and marrow lymphocytes as related to lymphopoiesis and hematopoiesis. Blood 1970, 35:257–271
130. Bullock WW, Anderson D, Golding B: Sex steroid influences on delayed type hypersensitivity (DHT) in Lewis (LEW) rats (Abstr). Fed Proc 1980, 39:1136
131. Wyle FA, Kent JR: Immunosuppression by sex steroid hormones. Clin Exp Immunol 1977, 27:407–415
132. Skinnider LF, Laxdal V: The effect of progesterone, oestrogens and hydrocortisones on the mitogenic response of lymphocytes to phytohaemagglutinin in pregnant and nonpregnant women. Br J Obstet Gynaecol 1981, 88: 1110–1117
133. Lawrence R, Church JA, Richards W, Borzy M: Immunological mechanisms in the maintenance of pregnancy. Ann Allergy 1980, 44:166–168
134. Clemens LE, Sitteri PK, Stites DP: Mechanism of immunosuppression of progesterone on maternal lymphocyte activation during pregnancy. J Immunol 1979, 122:1978–1985
135. Mendelson J, Multer MM, Bernheim JL: Inhibition of human lymphocyte stimulation by steroid hormones: Cytokinetic mechanisms. Clin Exp Immunol 1977, 278:127–132
136. Stimson WH: Are pregnancy-associated proteins responsible for the inhibition of lymphocyte transformation by pregnancy serum? Clin Exp Immunol 1980, 40: 157–163
137. Toder V, Blank M, Nebel L: Immunoregulatory mechanisms in pregnancy. Transplantation 1982, 33:41–63
138. Murgita RA: The immunosuppressive role of alphafetoprotein during pregnancy. Scand J Immunol 1976, 5:1003–1007
139. Yohn DS: Sex related resistance in hamsters to adenovirus oncogenesis. Prog Exp Tumor Res 1973, 18:138–145
140. Kenny JF, Gray JA: Sex differences in immunologic response: Studies of antibody production by individual spleen cells after stimulus with Escherichia coli antigen. Pediatr Res 1971, 5:246–255
141. Aycock WL: Alterations in autoarceologic susceptibility to experimental poliomyelitis. Proc Soc Exp Biol 1936, 34:573–574
142. Thompson JS, Crawford MK, Reilly RW, Severson CD: The effect of estrogenic hormones on immune responses in normal and irradiated mice. J Immunol 1967, 98:331–335
143. Ablin RJ: Modulatory effects of estrogen on immunologic responsiveness. Am J Rep Immunol 1981, 1:206–209

144. Ablin RJ, Kalland T: Immunomodulatory effects of oestrogen. J Immunol 1984, 132:3229
145. Mueller MN, Kappas A: Estrogen pharmacology. Proc Soc Exp Biol Med 1964, 117:845-847
146. Talal N, Roubinian JR: Sex hormones and autoimmunity, Genetic Control of Autoimmune Disease. Edited by NR Rose, PE Bigazzi, NL Warner. New York, Elsevier/North Holland, 1978, pp 163-176
147. Bick PH, Tucker AN, White KL Jr, Holsapple MP: Effects of subchronic exposure to diethylstilbesterol on humoral immune function in adult female (C3B6) Fl mice. Immunopharmacology 1984, 7:27-39
148. Mathur S, Mathur RS, Dowda H, Williamson HO, Faulk WP, Fudenberg HH: Sex steroid hormones and antibodies to candida albicans. Clin Exp Immunol 1978, 33:79-87
149. Hulka JF, Mohr K, Lieberman MW: Effect of synthetic progestational agents on allograft rejection and circulating antibody production. Endocrinology 1965, 77:897-903
150. Anderson JM: Transplantation: Nature's success. Lancet 1971, 2:1077-1082
151. Kind FA, Ciaccio LA: Suppression of immune response by progesterone. Endocrinol Exp 1980, 14:27-32
152. Mori T, Kobayashi H, Nishimoto H, Suzuki A, Nishimura T, Mori TS: Inhibitory effect of progesterone and 20-hydroxypreg-4-en-3-one on the phytohaemagglutinin-induced transformation of human lymphocytes. Am J Obstet Gynaecol 1977, 127:151-159
153. Holdstock G, Chastenay BF, Krawitt EL: Effects of testosterone, oestradiol and progesterone on immune regulation. Clin Exp Immunol 1982, 47:449-456
154. Krohn PL: The effect of steroid hormones on the survival of skin homografts in the rabbit. J Endocrinol 1954, 11:78-83
155. Friedman SB, Grota LJ, Glasgow LA: Differential susceptibility of male and female mice to encephalomyocarditis virus. Infect Immun 1972, 5:637-644
156. Shafie SM, Grantham FH: Role of hormones in the growth and regression of human breast cancer cells (MCF-7) transplanted into athymic nude mice. J Natl Cancer Inst 1981, 67:51-56
157. Kappas A, Jones HEH, Roitt IM: Effects of steroid sex hormones on immunological phenomenon. Nature 1963, 198:902
158. Okayasu I, Kong YM, Rose NR: Effect of castration and sex hormones on experimental autoimmune thyroiditis. Clin Immunol Immunopath 1981, 20:240-245
159. Steinberg AD, Roths JB, Murphy ED, Steinberg RT, Raveche ES: Effects of thymectomy or androgen administration upon the autoimmune disease of MRL/Mp-lpr/lpr mice. J Immunol 1980, 125:871-873
160. Shear HL, Wofsy D, Talal N: Effects of castration and sex hormones on immune clearance and autoimmune disease in MRL/Mp-lpr/lpr and MRL/Mp − +/+ mice. Clin Immunol Immunopathol 1983, 26:361-369
161. Dumont F, Habbersett RC, Monier JC: Thymic abnormalities induced by castration in aged (NZB X SJL) Fl male mice: Distinct effects of early and late castration. Clin Immunol Immunopathol 1983, 27:56-64
162. Lucas JA, Ansar Ahmed S, Casey ML, MacDonald PC: The prevention of autoantibody formation and prolonged survival in NZB/NZW F_1 mice fed dehydroisoandrosterone. J Clin Invest 1985, 2091-2093
163. Arnason BG, Richman DP: Effect of oral contraceptives on experimental demyelinating disease. Arch Neurol 1969, 21:103-106
164. Ahn YS, Harrington WJ, Simon SR, Mylvaganam R, Dall LM, So AG: Danazol for the treatment of idiopathic thrombocytopenic purpura. N Engl J Med 1982, 808:1396-1399
165. Pariser K, Gell J, Galfand J, Truksoy N, Agnello V: Pilot study in the use of danazol in treatment of patients with SLE (Abstr). Fifteenth International Congress of Rheumatology, 1981, p 483
166. Roubinian JR, Talal N, Greenspan JS, Goodman JR, Nussenzweig V: Danazol's failure to suppress autoimmunity in NZB/NZW Fl mice. Arthritis Rheum 1979, 22:1399-1402
167. Medawar PB, Sparrow EM: Effects of adrenocortical hormones, adrenocorticopropic hormone and pregnancy on skin transplantation immunity in mice. J Endocrinol 1956, 14:240-256
168. Kandil O: Role of the female sex hormones on the immunologic behavior in rabbits isoimmunized with semen. In J Fertil 1970, 15:77-87
169. Simmons RL, Price AL, Ozerkis AJ: The immunologic problem of pregnancy. Am J Obstet Gynecol 1968, 100:908-911
170. Liao S: Cellular receptors and mechanism of action of steroid hormones. Int Rev Cytol 1975, 41:87-172
171. Cooper EL (ed): Stress, Immunity and Aging. New York, Marcel Dekker, 1984
172. Siiteri PK: Sex hormone production and action. Arthritis Rheum 1979, 22:1284-1294
173. Calzolari A: Recherches experimentales sur un rapport probable entre la function du thymus et celle des testicules. Arch Ital Biol 1898, 30:71-89
174. Hammar JA: Uber gewicht involution und persistenz der thymus im post-fetal-leben des menschen. Arch F Anta U Physiol Anat Abt Supple Bd 1906, p 91
175. Chiodi H: The relationship between the thymus and the sexual organs. Endocrinology 1940, 26:107-116
176. Sobhon P, Jirasattham C: Effect of sex hormones on the thymus and lymphoid tissue of ovariectomized rats. Acta Anat 1974, 89:211-225
177. Allen LS, McClure JE, Goldstein AL, Barkley MS, Michael SD: Estrogen and thymic hormone interactions in the female mouse. J Rep Immunol 1984, 61:25-37
178. Hattori M, Brandon MR: Thymus and the endocrine system. J Endocrinol 1983, 83:101-107
179. Dumont F, Barrois R, Habbersett RC: Prepubertal orchidectomy induces thymic abnormality in aging (NZB × SJL/J) mice. J Immunol 1982, 129:1642-1648
180. Nishizuka Y, Sakakura Y: Ovarian dysgenesis induced by neonatal thymectomy in the mouse. Endocrinology 1971, 89:886-893
181. Michael SD, Taguchi O, Nishizuka Y: Effect of neonatal thymectomy on ovarian development and plasma LH, FSH, GH and PRL in the mouse. Biol Reprod 1980, 22:343-350
182. Rebar RW, Mujake A, Low TLK, Goldstein AL: Thymosin stimulates secretion of luteinizing hormone-releasing factor. Science 1981, 214:669-671
183. Rebar RW, Miyaka A, Erickson GF, Law TLK, Goldstein AL: The influence of the thymus gland on reproductive function, Factors Regulating Ovarian Function. Edited by GS Greenwald, PF Teranova. New York, Raven Press, 1983, pp 465-470
184. Michael SD, Taguchi O, Nishizuka Y, McClure JE, Goldstein AL, Barkley MS: The effect of neonatal thymectomy on early follicular loss and circulating levels of cortisone, progesterone, estradiol and thymosin $\alpha 1$, Dynamics of Ovarian Function. Edited by N Schwartz, M Hunzicker-Dunn. New York, Raven Press, 1983, pp 279-284
185. Talal N, Roubinian JR: Sex hormones and autoimmunity, Genetic Control of Autoimmune Diseases. Edited by NR Rose, PE Bigazzi, NL Warner. New York, Elsevier/North-Holland, 1978, pp 163-176
186. McCruden AB, Stimson WH: Androgen binding cytosol receptors in the rat thymus. Thymus 1981, 3:105-117
187. Grossman CJ, Sholiton LJ, Helmsworth JA: Characteristics of the cytoplasmic and nuclear dihydrotestoster-

one receptors of human thymic tissues. Steroids 1983, 42:11–22
188. Kalland TJ: Alterations of antibody response in female mice after neonatal exposure to diethylstilbestrol. J Immunol 1980, 24:194–198
189. Stumpf WE, Sar M: Autoradiographic localization of estrogen, androgen, progestin and glucocortiscosteroid in "target tissues" and "nontarget tissues," Receptors and Mechanisms of Action of Steroid Hormones. New York, Marcel Dekker, 1976, p 41
190. Weaker FJ, Sheridan PJ: Autoradiographic localization of sex steroid hormones in the lymphatic organs of baboons. Cell Tissue Res 1983, 231:593–601
191. Novotny EA, Raveche ES, Sharrow S, Ottinger M, Steinberg AD: Analysis of thymocyte subpopulations following treatment with sex hormones. Clin Immunol Immunopathol 1983, 28:205–217
192. Birkeland SA, Kristofferson K: Cellular immunity in pregnancy. Clin Exp Immunol 1977, 30:408–412
193. Tallon DF, Corcoran DJD, O'Dwyer EM, Greally JF: Circulating lymphocyte subpopulations in pregnancy. J Immunol 1984, 132:1784–1787
194. Cohen JHM, Daniel L, Cordier G, Saez S, Revillard JP: Sex steroid receptors in peripheral T cells. J Immunol 131:2767–2771
195. Ranelletti FO, Carmignani M, Marchetti P, Natoli C, Iacobelli S: Estrogen binding by neoplastic human thymus cytosol. Eur J Cancer 1980, 16:951–956
196. Paavonen T, Andersson LC, Adlercreutz H: Sex hormone regulation of *in vitro* immune responses. J Exp Med 1981, 154:1935–1945
197. Dauphinee MJ, Kipper S, Wofsy D, Talal N: Interleukin-2 deficiency is a common feature of autoimmune mice. J Immunol 1981, 127:2483–2487
198. Talal N, Dauphinee MJ, Wofsy D: Interleukin-2 deficiency, genes and systemic lupus erythematosus. Arthritis Rheum 1982, 25:838–842
199. Talal N: Interferon and natural killer cells in rheumatic diseases. Immunology of Rheumatic Diseases. Edited by S Gupta, N Talal. New York, Plenum Publishing, 1985
200. Herberman RB, Ortaldo JR, Timonen T, Reynolds CW, Djeu JY, Pestka S, Stahton J: Interferon and natural killer (NK) cells. Texas Reports on Biol Med 1981/82, 41:590–595
201. Seaman WE, Talal N: The effect of 17β-estradiol on natural killing in the mouse, Natural cell-mediated immunity against tumors. Edited by RB Herberman. New York, Academic Press, 1980, p 765
202. Kalland T: Exposure of neonatal female mice to diethylstilbesterol persistently impairs NK activity through reduction of effector cells at the bone marrow level. Immunopharmacology 1984, 7:127–134
203. Kalland T, Campbell T: Effects of diethylstibesterol on human natural killer cells *in vitro*. Immunopharmacology 1984, 8:19–25
204. Moore MAS, Owen JTT: Experimental studies on the development of the bursa of Fabricius. Dev Biol 1966, 14:40–51
205. Gasc JM, Sar M, Stumpf WE: Androgen target cells in bursa of Fabricius of the chick embyro: Autoradiographic localization. Proc Soc Exp Biol Med 1979, 160:55–58
206. Nicol T, Bilbey DLJ, Charles LM, Cordingley JL, Roberts VB: Oestrogen: The natural stimulant of body defense. J Endocrinol 1964, 30:277–291
207. Kelly LS, Brown BA, Dobson EL: Cell division and phagocytic activity in liver reticuloendothelial cells. Proc Soc Exp Biol Med 1962, 110:555–559
208. Dulk MMCD, Crofton RW, VanFurth R: Origin and kinetics of Kupffer cells during an acute inflammatory response. Immunology 1979, 37:7–14
209. Boorman GA, Luster MI, Dean JH, Wilson RE: The effect of adult exposure of diethylstilbesterol in the mouse on macrophage function and numbers. J Reticuloendothel Soc 1980, 28:547–559
210. Dean JH, Boorman GA, Luster MI, Adkins B, Laver LD, Adams DO: Effects of agents of environmental concern on macrophage function, Mononuclear Phagocyte Biology. Edited by A Volkman. New York, Marcel Dekker, 1984
211. Besodovsky HO, Del Rey AE, Sorkin E: What do the immune system and the brain know about each other? Immunol Today 1983, 4:342–346
212. Ansar Ahmed S, Talal N: The survival value of nonclassic target sites for sex hormone action in the immune and central nervous system. Clin Immunol Newsletter 1985, 6:97–99
213. Luster MI, Boorman GA, Korach KS, Dieter MP, Hong L: Myelotoxicity resulting from exogeneous estrogens: evidence for biomodal mechanisms of action. Int J Immunopharmacol 1984, 6:287–298
214. Blackwell CJ, Flower RJ: Effects of steroid hormones on tissue levels of prostaglandin 15-hydroxydehydrogenase in the rat. Br J Pharmacol 1976, 56:343–344
215. Casey ML, Hurd ER, Gilliam JN, MacDonald PC, Johnston JM: NAD^+-dependent 15-hydroxyprostaglandin dehydrogenease activity in kidney tissue from NZB/NZW F_1 hybrid mice. Immunology 1982, 45:23–26
216. Bienenstock J, Befus AD: Mucosal immunology. Immunology 1980, 41:249–270
217. Sullivan DA, Underdown BJ, Wira CR: Steroid hormone regulation of free secretory component in the rat uterus. Immunology 1983, 49:379–386
218. Sullivan D, Bloch KJ, Allansmith MR: Hormonal influence on the secretory immune system of the eye. J Immunol 1984, 132:1130–1135
219. Parker M, Gene regulation. Nature 1983, 304:687–688
220. Nagayama Y, Fukase M: Suppression of ^3H-thymidine incorporation into PHA-stimulated lymphocytes by testosterone. Acta Haematol Jpn 1978, 41:11–16